

DESCRIPTION

TEST PIECE FOR PROTEIN ASSAY, AND METHOD FOR MANUFACTURING
THE SAME

5

TECHNICAL FIELD

This invention relates to a test piece for protein
assay for assaying proteins present in protein-containing
samples (body fluid such as blood and urine, or protein-
10 containing beverages, or factory wastewater, etc.), and also
relates to a method for manufacturing such a test piece.

BACKGROUND ART

Assaying the protein in a biological sample is
15 important in pathological diagnosis. For instance, the
amount of serum albumin decreases in the case of diminished
liver function, while the amount of protein in urine
increases in the case of nephritis, nephrotic syndrome,
lithiasis, tumors, other such kidney and urinary tract
20 disorders, disorders of the circulatory system and disorders
of central nervous system. Therefore, assaying albumin or
other proteins can be an important clue in the diagnosis of
these disorders.

A simple assay method featuring the use of a protein
25 error indicator is known in the field of protein assay.
With this assay method, tetrabromophenol blue (TBPB) is used,
for instance, as the protein error indicator. As one
example, urine test paper made with TBPB is widely used for

primary screening purposes. TBPB changes from yellow to blue through the dissociation of phenolic hydroxyl groups at a pH of about 3 when a protein is present, and therefore can be used to detect protein.

5 However, test paper made using TBPB as the indicator has inadequate sensitivity with respect to the low protein concentrations of 10 to 20 mg/dL required for clinical use, and is therefore sometimes incapable of detecting protein accurately. For example, in a visual evaluation conducted
10 by comparison with a color chart, the color is very similar between negative protein and trace protein, making it difficult to tell the two apart and hampering accurate evaluation. Meanwhile, when a urine test paper assay apparatus is used, the low sensitivity of TBPB often results
15 in erroneous evaluation.

Consequently, there has been a need for a technique that would allow low concentrations of protein to be quantified at higher sensitivity.

20 DISCLOSURE OF THE INVENTION

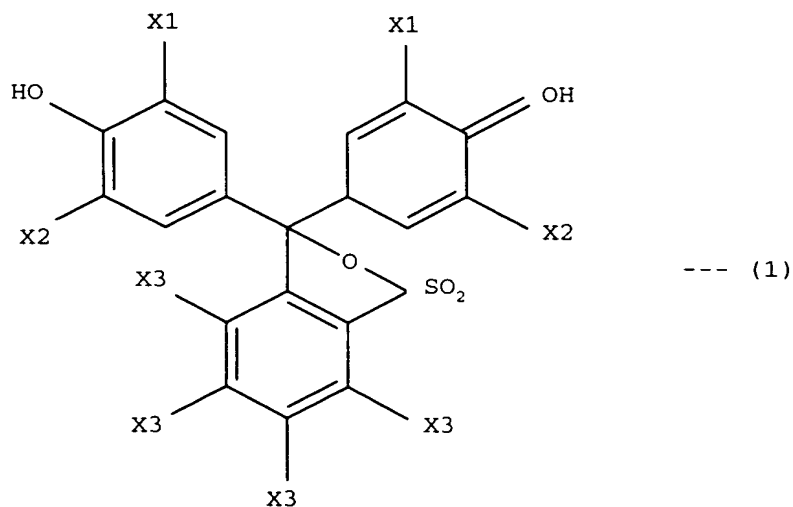
As a result of diligent research aimed at solving the above problems, the inventors arrived at the present invention upon discovering that low concentrations of protein can be assayed at high sensitivity if the test piece
25 contains a surfactant.

Specifically, the test piece for protein assay provided by a first aspect of the present invention is a test piece for protein assay that is used for quantifying or semi-

quantifying a protein and that contains an acidic pH indicator, containing a surfactant as a sensitizer for increasing coloration sensitivity to protein.

A second aspect of the present invention is a method
5 for manufacturing a test piece for protein assay that is used for quantifying or semi-quantifying a protein, by impregnating an absorbent carrier with an impregnant containing an acidic pH indicator and a sensitizer, and then drying this product, wherein a surfactant is used as the
10 sensitizer.

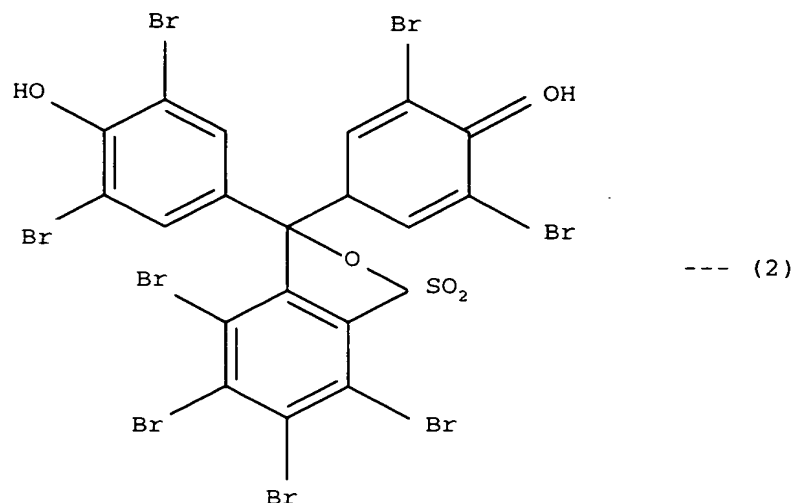
An example of an acidic pH indicator that can be used in the present invention is a triphenylmethane-based indicator. A typical example of a triphenylmethane-based indicator is expressed by the following Chemical Formula (1).



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In Chemical Formula (1), X1 is a halogen, a nitro group, or a nitroso group, and X2 and X3 are the same or different halogens.

With the present invention, it is preferable to use the tetrabromophenol blue (TBPB) expressed by the following Chemical Formula (2) as the triphenylmethane-based indicator.



5 There are no particular restrictions on the concentration of the acidic pH indicator (such as TBPB) in the impregnant, but it is typically 0.1 to 5 mM, and preferably 0.3 to 1 mM.

It is preferable to use a cationic surfactant as the
10 surfactant (sensitizer). In addition to this, or instead of this, a nonionic surfactant may be used.

A quaternary ammonium salt can be used, for example, as the cationic surfactant. This quaternary ammonium salt can be an alkyltrimethylammonium salt, benzalkonium salt,
15 salt, dialkyldimethylammonium salt, benzalkonium salt, imidazolium salt, or the like. An aliphatic amine salt can also be used as the cationic surfactant.

The nonionic surfactant can be either an ether type, ether ester type, ester type, or nitrogen-containing type.

Examples of ether-type surfactants include polyoxyethylene alkyl ether, polyoxyethylene secondary alcohol ether, polyoxyethylene alkylphenyl ether, polyoxyethylene sterol ether, polyoxyethylene lanolin
5 derivative, ethylene oxide derivative of alkylphenol formalin condensate, polyoxyethylene polyoxypropylene block polymer, and polyoxyethylene polyoxypropylene alkyl ether.

Examples of ether ester-type surfactants include polyoxyethylene glycerol fatty acid ester, polyoxyethylene
10 castor oil, polyoxyethylene hydrogenated castor oil, polyoxyethylene sorbitan fatty acid ester, and polyoxyethylene sorbitol fatty acid ester.

Examples of ester-type surfactants include polyethylene glycol fatty acid ester, fatty acid monoglyceride,
15 monoglycerol fatty acid ester, sorbitan fatty acid ester, propylene glycol fatty acid ester, and sucrose fatty acid ester.

Examples of nitrogen-containing surfactants include fatty acid alkanolamide, polyoxyethylene fatty acid amide,
20 polyoxyethylenealkylamine, and alkylamine oxide.

The cationic surfactant used in the present invention is typically benzyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, lauryltrimethylammonium bromide, or zephiramine, and the nonionic surfactant is
25 typically polyethylene glycol. Naturally, a plurality of different surfactants may be used together, in which case it is preferable to use a combination of a cationic surfactant and a nonionic surfactant. Typically, a combination of

benzyltrimethylammonium bromide, which is a cationic surfactant, and polyethylene glycol, which is a nonionic surfactant, is used.

There are no particular restrictions on the
5 concentration of the sensitizer (surfactant) in the impregnant, but it is typically 0.01 to 5 wt%, and preferably 0.01 to 1 wt%.

A polycarbonate, polyvinyl alcohol, or other such polymer material may also be used in addition to the
10 surfactant as a sensitizer.

The pH of the impregnant is set to be somewhat lower than the pKa of the acidic pH indicator. When TBPB is used as the acidic pH indicator, the pH of the impregnant is to be from 2.0 to 4.5, and preferably 2.0 to 3.5.

Any buffer can be used as long as it has a good
15 buffering action at the pH of the impregnant (between 2.0 and 4.5, for instance) and does not impede the reaction between the acidic pH indicator and the protein. Examples of buffers that can be used include glycine buffer, citrate
20 buffer, succinate buffer, malate buffer, and tartrate buffer. There are no particular restrictions on the concentration of the buffer in the impregnant, but it is typically from 0.1 to 1.5 M, and preferably 0.3 to 1 M.

A porous substance that contains no protein component
25 can be used as the absorbent carrier, and can be used in the form of a sheet or film, for example. Examples of porous substances include paper-like materials, foams, woven materials, nonwoven materials, and knits. Examples of the

material used to form the absorbent carrier include cotton, linen, cellulose, nitrocellulose, cellulose acetate, rock wool, glass fiber, silica fiber, carbon fiber, boron fiber, polyamide, aramid, polyvinyl alcohol, polyvinyl acetate, rayon, polyester, nylon, polyacrylic acid, polyacrylic ester, and polyolefin. There are no particular restrictions on the shape of the absorbent carrier, but it is generally rectangular (either short and wide or long and narrow), circular, or oval.

The test piece for protein assay of the present invention can be used directly as it is, or after first being bonded to a non-absorbent material.

The non-absorbent material is used in the form of a sheet or film, for example. Examples of the material used to form this non-absorbent material include polyethylene terephthalate, polyester, polypropylene, polyethylene, polyvinyl chloride, polyvinylidene chloride, and polystyrene.

EXAMPLES

[Example 1]

In this example, each test piece was impregnated with urine having an albumin concentration of 0.3 mg/dL (negative) or 15 mg/dL (positive), and the reflectance of the test piece was measured. The test pieces were formed by impregnating filter paper (3MMChr made by Whatman) with an impregnant, and then drying. The impregnant was produced by adding 0.2 wt% benzyltrimethylammonium bromide (a cationic surfactant) as a sensitizer to the base composition shown in

Table 1. Reflectance was measured with a colorimeter at a measurement wavelength of 630 nm. The measurement results are given in Table 2.

[Example 2]

5 In this example, reflectance was measured in the same manner as in Example 1, except that the impregnant was produced by adding hexadecyltrimethylammonium bromide (a cationic surfactant) as a sensitizer in an amount of 0.01 wt% to the base composition in Table 1. The
10 measurement results are given in Table 2.

[Example 3]

 In this example, reflectance was measured in the same manner as in Example 1, except that the impregnant was produced by adding lauryltrimethylammonium bromide (a
15 cationic surfactant) as a sensitizer in an amount of 0.01 wt% to the base composition in Table 1. The measurement results are given in Table 2.

[Example 4]

 In this example, reflectance was measured in the same
20 manner as in Example 1, except that the impregnant was produced by adding polyethylene glycol (a nonionic surfactant) as a sensitizer in an amount of 0.5 wt% to the base composition in Table 1. The measurement results are given in Table 2.

25 [Example 5]

 In this example, reflectance was measured in the same manner as in Example 1, except that the impregnant was produced by adding benzyltrimethylammonium bromide (a

cationic surfactant) in an amount of 0.2 wt% and polyethylene glycol (a nonionic surfactant) in an amount of 0.5 wt% as sensitizers to the base composition in Table 1. The measurement results are given in Table 2.

5 [Example 6]

In this example, reflectance was measured in the same manner as in Example 1, except that the impregnant was produced by adding lauryltrimethylammonium bromide (a cationic surfactant) in an amount of 0.01 wt% and
10 polyethylene glycol (a nonionic surfactant) in an amount of 0.5 wt% as sensitizers to the base composition in Table 1. The measurement results are given in Table 2.

[Example 7]

In this example, reflectance was measured in the same
15 manner as in Example 1, except that the impregnant was produced by adding zephiramine (a cationic surfactant) in an amount of 0.01 wt% and polyethylene glycol (a nonionic surfactant) in an amount of 0.5 wt% as sensitizers to the base composition in Table 1. The measurement results are
20 given in Table 2.

[Comparative Example 1]

In this comparative example, reflectance was measured in the same manner as in Example 1, except that the impregnant was the base composition itself (containing no
25 surfactant (sensitizer)). The measurement results are given in Table 2.

Table 1: Base composition

Indicator	Buffer (pH 3.4)	Solvent
TBPB 0.5 mM	malate buffer 0.8 M	ethanol 30 wt%

Table 2: Measurement results

	Sensitizer	Reflectance (%)		Differential Δ (%)
		0.3 mg/dL (negative)	15 mg/dL (positive)	
Ex. 1	benzyltrimethylammonium bromide 0.2 wt%	60.7	39.3	21.4
Ex. 2	hexadecyltrimethylammonium bromide 0.01 wt%	60.4	40.6	19.8
Ex. 3	lauryltrimethylammonium bromide 0.01 wt%	59.0	38.7	20.3
Ex. 4	polyethylene glycol 0.5 wt%	57.0	40.4	16.6
Ex. 5	benzyltrimethylammonium bromide 0.2 wt% polyethylene glycol 0.5 wt%	60.6	38.3	22.3
Ex. 6	lauryltrimethylammonium bromide 0.01 wt% polyethylene glycol 0.5 wt%	59.1	35.9	23.2
Ex. 7	zephiramine 0.01 wt% polyethylene glycol 0.5 wt%	61.2	41.5	19.8
C. E. 1	none	60.7	48.9	11.8

5 As is clear from Table 2, reflectance at an albumin concentration of 15 mg/dL was lower when a surfactant was contained than when no surfactant was contained. Accordingly, the differential Δ (%) between negative (0.3 mg/dL) and positive (15 mg/dL) when a surfactant was

10 contained was about twice that when no surfactant was contained. We can therefore conclude that when a surfactant is contained, sensitivity to albumin is higher, and albumin can be properly detected even when the albumin concentration is low (about 10 to 20 mg/dL). The albumin detection

15 sensitivity was particularly high when a cationic surfactant was used alone and when it is used together with a nonionic surfactant. In this respect, a cationic surfactant can be considered to be preferable as a sensitizer.

The experiment results in these examples pertain to urine samples, but the present invention is not limited to urine, and can also be applied to the quantification of protein in any of various other samples containing protein,
5 such as blood, protein-containing beverages, and factory wastewater.